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DNA Microarray Technology

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DNA Microarray Technology

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Abstract

Collaboration between Sandia National Laboratories and the University of New Mexico Biology Department resulted in the capability to train students in microarray techniques and the interpretation of data from microarray experiments. These studies provide for a better understanding of the role of stationary phase and the gene regulation involved in exit from stationary phase, which may eventually have important clinical implications. Importantly, this research trained numerous students and is the basis for three new Ph.D. projects.

Introduction

Scientists at Sandia National Laboratories and at the University of New Mexico have collaborated to bring DNA microarray technologies to the University, and to develop new technologies based on DNA microarrays. This LDRD project involved the construction of a microarrayer, the evaluation of RNA preparation techniques, the evaluation of microarray printing tips and sample preparation processes. The work also involved novel new analysis methods. The arrayer is fully functional, a satisfactory RNA preparation kit has been identified, and problems with printing tips have been solved. With this functioning equipment and laboratory techniques, we have been able to print microarrays with the entire genome for *Saccharomyces cervisae*. We have conducted two major microarray experiments addressing whole genome expression levels in *S. cervisae* as it exits stationary phase. The surprising results indicate that stationary phase may not be a part of the normal cell cycle, as had been widely assumed [Werner-Washburne 2001]. This project equipped a functioning research laboratory, trained five students and led to three new Ph.D. projects. The results have been presented at an international conference [Werner-Washburne 2001, Prague] and have been submitted for publication in a leading, peer-reviewed journal.

Activities

This three year LDRD project that had multiple objectives. The most important objective was to establish research collaborations between Sandia National Laboratories and the University of New Mexico Biology Department. The second was to enable local microarray experiments and to develop the methods and process controls necessary to achieve high quality results. The final objective was to research the interesting issues of gene expression during and immediately following exit from stationary phase in the yeast *S. cerevisiae.*

The collaboration between Sandia National Laboratories and the University of New Mexico has been quite successful. Importantly, we jointly developed the ability to conduct and analyze micorarray experiments using both commercial gene array membranes and our own arrays printed on glass slides. We have analyzed these experiments with commercially available software (for example, Gene Spring), and with VxInsight, which was developed at Sandia National Laboratories. This collaboration has resulted in improvements to the VxInsight software, joint publications and numerous presentations (see below). Importantly, it has exposed many students to researchers and research opportunities that would not have been possible without this project.

One of the most important elements of this project was the evaluation and selection of microarray printing tips, various varieties of slides (with and without DNA covalent bonding), and the identification of appropriate RNA processing methods. We evaluated three sources of printing tips, three varieties of slides, numerous conditions for obtaining optimum binding of DNA to the slides, and three RNA preparation kits. As a result of this careful evaluation, we can reliably print the entire genome for *S. cerevisiae* on glass

slides for use in continuing research programs. The ability to print whole genomes is a particularly important capability because there is no commercial source for microarrays for the genomes of many of the organisms studied at the University of New Mexico, so that they must be prepared locally.

Results

Figure 1 shows the microarrayer built in the first year of the project. Figure 2 shows the print head with the fragile tips that must actually impact on the glass slides in order to deposit DNA. Figure 3 shows the results of tests of three different types of glass-slide surfaces and two different types of spotting solutions. Figure 4 shows the results of a test to determine the binding of DNA to a Corning GMT-I slide over a period of 72 hours. Figure 5 shows the results of a test to determine the best post-spotting treatment to retain DNA on the slide. Through these experiments, we have been able to determine the conditions that are best suited for making stable arrays.

Figure 1. The microarray printer. Jose Weber, an undergraduate who will attend graduate school next fall**,** prints arrays. Jose was one of the students who helped build the arrayer and is co-author on one of the papers that has come from this collaboration.

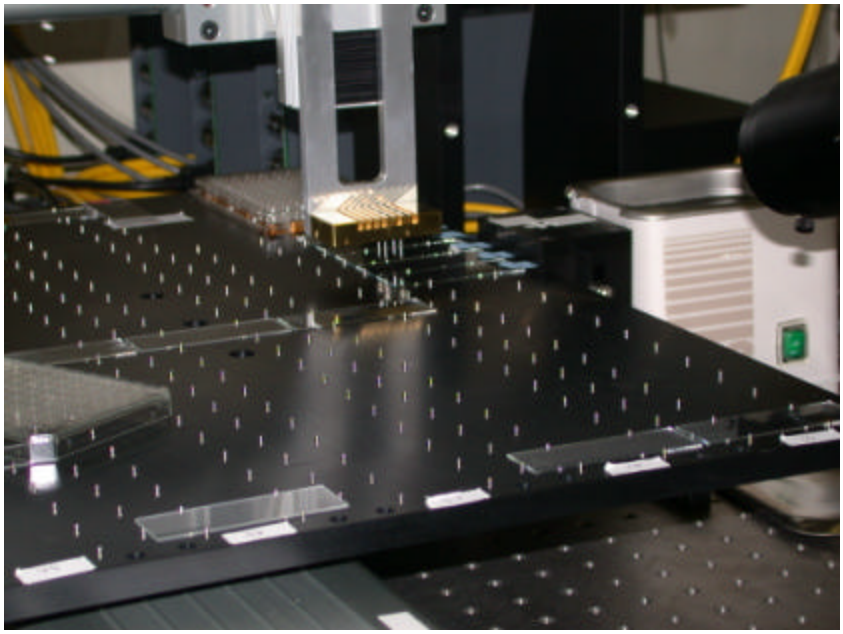

Figure 2. The print head lowering the printing tips, which actually contact the glass slides. The arrayer is capable of printing 100 glass slides at one time. We are currently testing whether cooling the printing platform enhances slide surface stability to allow for longer print runs.

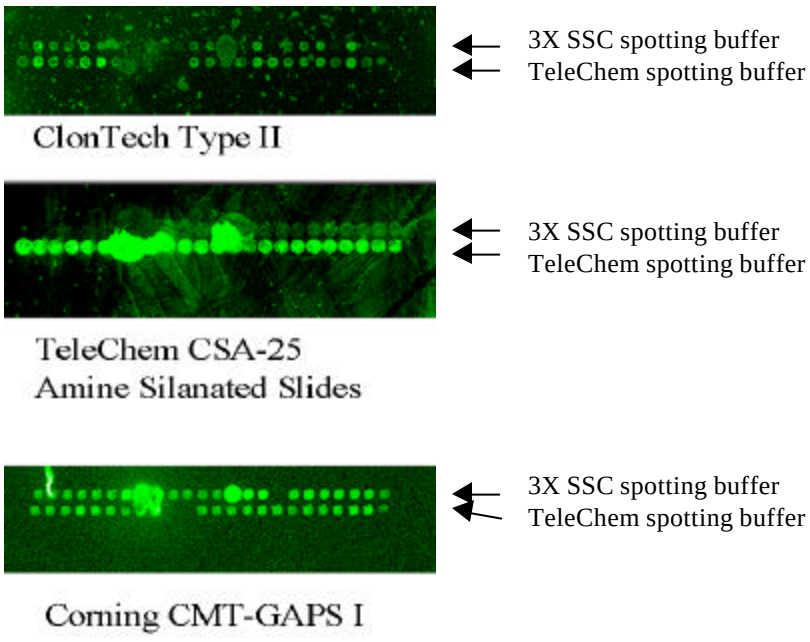

Figure 3 Tests of three different types of microarray slides and two different spotting solutions for binding DNA. Clonetech II, TeleChem, and Corning GMT-I slides were printed with yeast oligonucleotides (DNA) and incubated in a mock hybridization reaction. Spots containing 3XSSC are on the top line and those containing TeleChem spotting buffer are on the bottom line. DNA was visualized with SyberGreen dye. DNAs were solubilized and printed in 3XSSC (a buffered, salt solution) or TeleChem spotting buffer. We concluded from this test that Corning slides with TeleChem spotting buffer gave the best results.

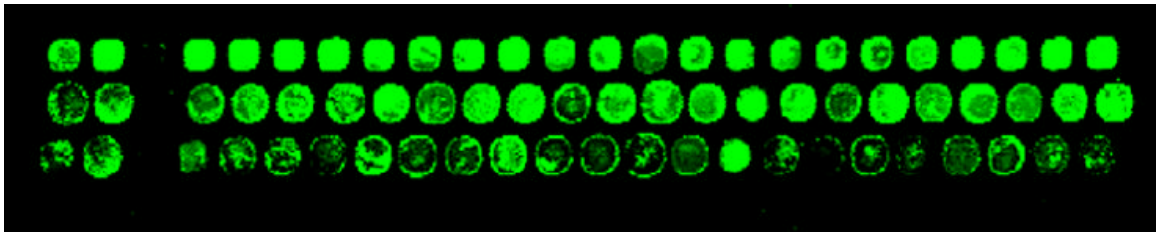

Figure 4. Test of the stability of the surface chemistry of Corning GMT-I slides. Slides were printed at day 1 (top line), 2 (second line), and 3 (bottom line) using yeast oligonucleotides. After the third day, slides were processed and mock-hybridized to test for DNA binding. We concluded from this that we needed to spot entire genomes in less than 24 hours. We are currently testing whether cooling the spotting platform will prolong the surface binding of these slides.

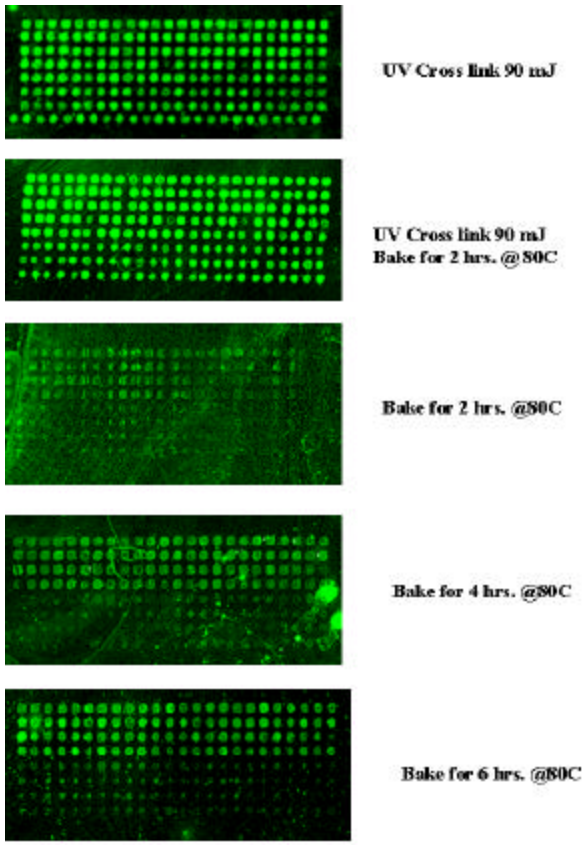

Figure 5. Test of post-spotting treatments for DNA retention. Slides were exposed to UV-crosslinking with or without 2 hours baking at 80^0C, or baked for 2, 4, or 6 hours without crosslinking. In each slide, the top four rows were printed on day1 and the bottom four lines were printed on day 2. We concluded that UV crosslinking follwed by 2 hours of baking gave the best results.

RNA preparation is a major source of error in the measurement process [Schena 2000]. We evaluated commercial RNA preparation kits from: Gentra, Tri-Reagent, and a combination of Gentra with Tri-reagent. The Gentra protocol worked best, giving the greatest yield and highest quality RNA (as determined by lack of DNA contamination and RNA degradation based on the observed degradation of ribosomal subunits). We did modify the typical Gentra protocol; our protocol is included in Appendix I.

Figure 6 shows the group of students trained with this equipment, and in these methods. What is not shown is the incredible energy and enthusiasm of these students. This group consists of undergraduates, graduate students, and post-doctorial researchers, all working together and sharing their research experience. All together, about 20 students have worked in this collaboration. Two students (both minorities) who graduated and are no longer in the group are working at the Institute for Systems Biology in Seattle and the Joint Genome Institute at Walnut Creek.

Figure 6 The Werner-Washburne Laboratory Group, June, 2001. Missing are Judith Galbraith (Ph.D. student) and Mark Fleharty

Figure 7 shows gene clustering with VxInsight [Davidson, 2001] in a comparison between normal cell cycle expressions [Spellman, 1998] and the expression levels observed in our experiments following exit from stationary phase. Notice that in the cell-cycle experiment the group of G1 genes, highlighted in Figure 6a, are no longer closely clustered together in clusters for the stationary phase experiment, shown in Figure 6b.

Note that several groups of genes, notably those most strongly associated with stationary phase (see cluster marked Stationary Phase in Figure 7a) and, also, those associated with ribosomal protein productions (see Ribosome Ridge in Figure 7b), cluster strongly in the stationary phase data. These clusters are tantalizingly important because many diseases exist for years in stationary phase before leaving stationary phase and entering an exponential growth phase to become life threatening. The identification of particular genes and proteins that are still active in stationary phase offers the possibility of identifying new, and novel drug targets that would be active against these diseases during their quiescent phase.

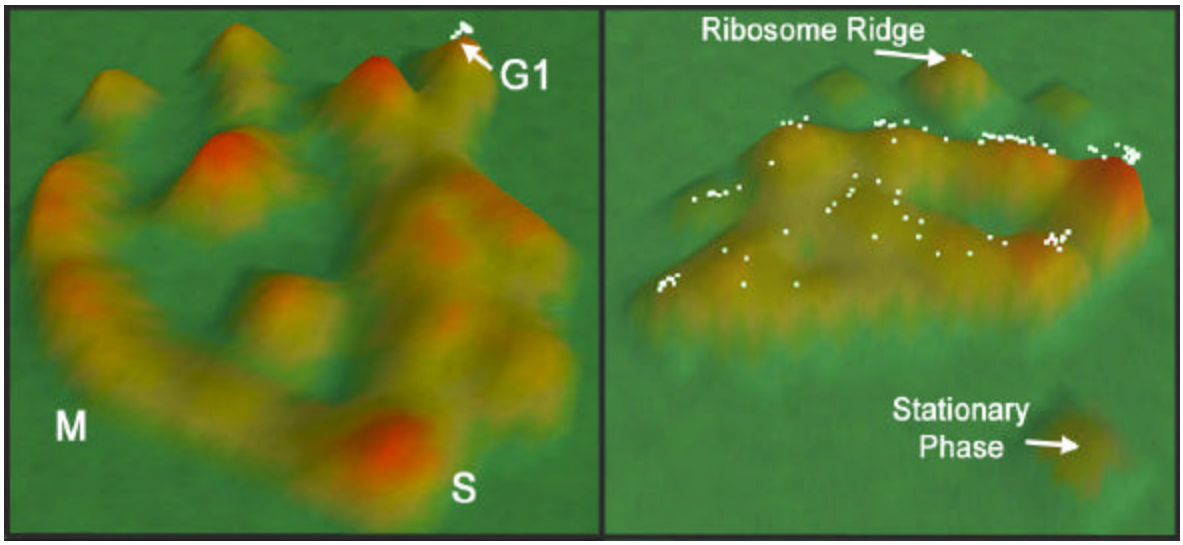

Figure 7a (left) and 7b (right) Gene expression during the normal cell cycle (left) and following exit from stationary phase in yeast (right).

Conclusions

This small LDRD project has been hugely successful. A fully functional microarray technology laboratory is in active use by many students, post-doctorial researchers and professors at the University of New Mexico. This research has allowed new insights into gene regulation, and may have identified important new drug targets. Significantly, three Ph.D. projects have resulted from this LDRD collaboration. The collaborations and the research based on this microarray capability are expected to continue to yield results in the future.

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Invited talks

Werner-Washburne, Margaret (2001 Prague), Stationary phase in yeast: A genomic analysis, International Conference on Yeast Genetics and Molecular Biology, Prague, Aug. 26-31, 2001.

Visual comparison of genome-scale datasets from yeast: Insights into cellular function.
The Institute for Genome Research (TIGR),
Rockville, MD; June 12, 2001

The Yin and Yang of Genomics: doing it - microarray analysis of stationary-phase yeast- and teaching it.
National Science Foundation; Arlington, VA; June 13, 2001

Exit from stationary phase in yeast: Gene expression analysis
Yeast Genetics and Molecular Biology Meeting
Seattle, WA; July 25-30, 2000

Genomics and its challenge for minority education
Zeta Phi Beta National Conference
Philadelphia, PA ; July 6-7. 2000

Genomics: A Revolution Before Our Eyes
AAAS SWARM meeting
Las Cruces, NM; April 10, 2000

Insight with VxInsight
Southwest Genomics and Biotechnology Alliance – Day of Talks I
Albuquerque, NM; March 22, 2000

An overview to genomics
Presentation to Functional Genomics Working Group
Albuquerque, NM; May 10, 1999

The Biological Revolution: Genomics and its Challenge for Minority Education
Zeta Foundation meeting on the Human Genome Project
New Orleans, LA; April 16, 1999

Other documents produced through this collaboration

Following the Coil of Life – Editorial in the Millennium Series
Albuquerque Tribune, January 1, 2000
http://biology.unm.edu/biology/maggieww/Public_HTML/followingthecoil.htm

Southwest Genomics and Biotechnology Alliance White Paper
(http://www.sandia.gov/swgaba), July 1999

Appendix I

Laboratory Protocol for RNA Preparations Prior to Hybridizing with cDNA Microarrays

RNA prep for microarrays
Werner-Washburne lab

9/17/01 -- Juanita's modification of:
wfuge
10/11/99

The following procedure uses a modified Gentra RNA protocol. The major differences to the Gentra procedure are:

- Cell lysis by glass beads and minibeadbeater (Biospec – www.biospec.com)
- $CHCl_3$: Phenol treatment to remove lipids, DNA and protein to improve UV quantitation. Pure RNA is critical for efficient cDNA synthesis. Although, DNA contamination should not be a problem during first strand synthesis its presence could yield inaccuracies during RNA quantitation and will reduce microarray slide hybridization.
- Qiagen Rneasy column to remove residual phenol
- DnaseI treatment

The following Ambion Tech Notes provide additional information, which can be obtained off the Web: #181, 159, 158, and Tips from the Bench #4 pg. 10.

Cell Lysis

1. Collect several backup samples for each experiment. Collect 15-20 ODU (Optical Density Units) for Exponential Phase cells and 30-40 ODU for Stationary Phase and Diauxic Shift cells per sample. With bead-beating, 28-40 ODU yeilds about 300 ug of total RNA. Store pellets at -70^{o}C. For some experiments, washing the pellet is not feasible if one is doing a very tight time course. Angelina and Juanita elected to immediately freeze the unwashed pellet in liquid nitrogen after pouring off the supernatant (media)

2. To frozen pellet add:
- 300ul of **cell lysis solution** (Gentra)
- 2ul of CAT mRNA (1ng/ul) (Only for Northern blots)

3. Let pellet thaw in lysis solution on ice. Continue to mix the pellet during thawing so that any lysed cells are exposed to lysis buffer. This assures good quality RNA. Transfer this thawed solution to a microfuge tube of your choice. Screw top tubes prevent leakage, but 1.6ml microfuge tubes allow better visibility. If you choose the 1.6ml flip top tubes, you must clear the lip of all beads and parafilm the top to prevent leakage. Bead beater must be modified with the adapter screw for the 1.6 ml tubes.

4. Add two scoopfuls (homemade with a PCR tube strip – about 480-500ml total) of baked glass beads, (0.5mm) preferably cold, (cold beads are difficult to handle because of condensation).

5. Use the bead beater (speed: 50X100 rpm) to lyse cells for 30 sec alternating with 30 sec on ice (Do this 6X). (If you have many samples, leaving them on ice for longer than 30 sec does not decrease your yield or quality)

6. Centrifuge for 3 min in refrigerated centrifuge. Decant supernatant to chilled, fresh eppendorf tube.

RNA precipitation:

1. Add 100ul of **protein-DNA precipitation** solution (Gentra) to supernatant.

2. Invert X10 and ice for 5 min.

3. Centrifuge X 3 min (refrigerated centrifuge).

4. Add supernatant to pre-chilled eppendorf tube containing 300ul of cold100% isopropanol (2-propanol).

5. Mix by inversion 50 times. (exact number of times is not crucial, I tend to lose count -- mix well)

6. Centrifuge X 3 min then remove supernatant. Ascertain presence of a pellet.

7. Resuspend pellet in 100ul of depc-H_2O.

CH_3Cl: Phenol Treatment

1. Add 100ul of CH_3Cl: Phenol (saturated 5:1; pH 4.7-Amresco) to RNA. Isoamyl alcohol encourages a tight interface, most phenol mixes contain it,

2. Vortex X 1 min then centrifuge 10 min. (this also encourages a tight interface)

3. Remove 80ul of aqueous phase to fresh eppendorf tube. Do not attempt to remove all of the aqueous phase sample because undesirable products from the organic phase may also be drawn up.

4. Back-extract remaining (~20ul) Ch_3Cl: Phenol sample: Add 80ul of depc-H_20 and repeat steps 2 and 3. (See Ambion Tech Note: 5:4 pg.10 for additional information)
5. (Step 5 is no longer used)
6. Combine aqueous phases (i.e. RNA) from steps 3 and 4.

6′. EtOH precipitate the RNA by adding:

- 0.1 volume of 5M NH_4Oac
- 2.5 volumes 100% EtOH

Precipitate overnight at –20°C. This is not crucial, but definitely helps get everything to precipitate. 1-2 hours at –20°C, and in a crunch, 15-30 min at –70°C works great too.

7. Centrifuge precipitated RNA X20 min in refrigerated centrifuge.

8. Remove supernatant and wash RNA pellet with 0.5 ml of 70% EtOH. Vortex X1 min or until pellet is loosened from bottom.

9. Centrifuge X10min and remove EtOH. Centrifuge additional 1min and remove residual EtOH.

10. Dry pellet 10-15min (inverted on paper towel) and resuspend in water for short term storage and for downstream reactions or for long term storage in **50ul** of **RNA Storage Solution** (Na Citrate, pH 6.4; Ambion). Note : Formamide, our typical storage method inhibits reverse transcription. Store at –70°C, or at –20°C for short term storage.

Quantitation

Quantitate all RNAs at the same time to reduce variability.

~~Quantitate by UV and formaldehyde gel electrophoresis.~~ This is not necessary at this point if you proceed to Qiagen cleanup.

Note

I chose not proteinase K treat due to downstream use considerations. If this is be sure to do Phenol: CH_3Cl to inactivate proteinase K.

Qiagen cleanup

Do the following steps, keep in mind that the Rneasy column can hold a maximum of 100 ug:

Rneasy Mini protocol for RNA cleanup: steps 1-3 (4/2000 handbook) There is no need to add Beta-mercaptoethanol, since, at this point, we are not using cell lysate but rather a pretty clean RNA prep. The lack of Beta-mercaptoethanol (B-me), an Rnase inhibitor, has been tested and shown to not affect the quality of the RNA, or reduce the quantity any less than 10%, (In the range of recovery variation) *as seen by two experiments.* If you choose to use β-me, do your work in the hood, and dispose of your β-me supernatants in an appropriate waste container.

Rnase-Free-DNase Set Protocol: steps D2-D8 (1/1999 protocol), This does not include steps 4-5 and step D1 respectively since we are using these kits for RNA that is already pretty clean. *We have found that g the same clean RNA in DEPC treated water versus in Nuclease Free water (Rneasy kit) gives a 260/280 ratio of 1.8 and 2.0 respectively. We think this is related to the pH of the water, as described in the Rneasy protocol book.*

Assay RNA by spectrophotometer and agarose gel.

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